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ORIGINAL RESEARCH

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Genetic factors increasing barley grain yields under soil waterlogging

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Abstract

In-crop soil waterlogging can be caused by extreme rainfall events, high ground water tables, excessive irrigation, lateral ground water flow, either individually or in concert, and together these factors inhibit potential grain yields. However, the extent to which yield is influenced by the timing and duration of waterlogging relative to crop phenology is unknown. To investigate this, we conducted a range of waterlogging treatments on modern barley genotypes differing in their waterlogging tolerance, with tolerance conferred through aerenchyma formation under oxygen deficit conditions. Experiment 1 was conducted in a controlled environment using four waterlogging treatments: waterlogging at Zadoks stage (ZS) 12.5 for 1 or 2 months (WL1 and WL2, respectively), waterlogging at ZS 15 for 2 months (WL3), and waterlogging initiated 1 day before heading for 15 days (WL4). Experiment 2 was conducted in the field with WL2. Averaged across experiments, yield was reduced by 35% in W1 to 52% in WL3 due to fewer spikes/m^2 and kernels/spike. WL4 had the greatest impact on yield (70% reduction) due to its effect on spikelet fertility and grain filling. Phenology was delayed 1–8 ZS at the end of waterlogging treatments, with the waterlogging-susceptible cultivar Franklin showing the greatest delays, and waterlogging tolerant genotypes (Macquarie+, TAMF169) capable of aerenchyma formation under waterlogging having the least delays (0–4 ZS). Genotypes with aerenchyma formation QTL (Macquarie+) showed nonsignificant yield reduction compared with nonwaterlogged controls, preventing 23% yield loss under early phenological waterlogging stress. Late growth stage waterlogging substantially reduced average final grain yield by 70%.

KEYWORDS

barley, development, grain yield, phenology, waterlogging

1 | INTRODUCTION

Crop waterlogging is increasingly a global problem due to increased frequencies of extreme climate events (Wollenweber, Porter, & Schellberg, 2003). Globally, excessive water and poor soil drainage constraints adversely affect ~10% of arable land area (Setter & Waters, 2003), with average annual economic losses caused by crop waterlogging amounting to tens of billions of US dollars from 2004 to 2013 (Hirabayashi et al., 2013). With climate change, more than 10% of agricultural regions will have greater risk of waterlogging due to higher frequencies and greater magnitudes of extreme rainfall events (Chang-Fung-Martel, Harrison, Rawnsley, Smith, & Meinke, 2017; Harrison, Tardieu, Dong, Messina, & Hammer, 2014; Hirabayashi et al., 2013).

Waterlogging is a 'wicked problem': the phenomenon is highly complex and multi-faceted. In field crop experimental trials, waterlogging driven by excessive rainfall or subsurface or lateral flooding may have poor reproducibility, because waterlogging-prone environments have considerable complexity, including variable dimensions of time, space, biology, and chemistry. Thus, methods with which such events are analyzed and quantified in a farming systems context require careful consideration (Harrison, Cullen, & Armstrong, 2017; Harrison, Cullen, & Rawnsley, 2016).

Barley crops (*Hordeum vulgare* L.) are currently cultivated in more than 100 countries for use as animal feed and human consumption (Zhou, 2009). Global barley production has diminished over the last two past decades, decreasing from 155 Mt tons in 2008–2009 to 142 Mt in 2017–2018 (Statista, 2020). Part of this decline is due to increased frequency of waterlogging and susceptibility of barley to waterlogging stress damage (Setter & Waters, 2003). In many contexts, improving crop tolerance to low–mild waterlogging is generally cost effective; however under severe waterlogging, combined agronomic, engineering and genetic solutions are needed (Manik et al., 2019).

Defined physiologically, waterlogging tolerance is the survival or maintenance of growth under waterlogging relative to nonwaterlogged conditions (Gibbs & Greenway, 2003; van der Moezel, Pearce-Pinto, & Bell, 1991). Oxygen deficiency in soil pores caused by waterlogging reduces root growth, leading to premature leaf senescence and tillering, inhibition of dry matter accumulation, and production of sterile florets. In combination, such effects stun kernel number and weight, penalizing grain yield (Masoni, Pampana, & Arduini, 2016; de San Celedonio, Abeledo, Brihet, & Miralles, 2016; de San Celedonio, Abeledo, & Miralles, 2014, 2018).

Agronomically, crop waterlogging tolerance relies on their ability to recover after the stress period and compensate sufficiently to produce acceptable grain yield (Setter & Waters, 2003). Past studies have measured yield declines of 40%–79% in waterlogged barley, depending on genotype, growth stage, soil type, and duration of waterlogging (de San Celedonio, Abeledo, & Miralles, 2014). Yield loss in barley is also likely to be sensitive to the phenological stage with which waterlogging occurs (de San Celedonio et al., 2014). One of the few reports that examined the relationship between yield loss and phenological stage reported that barley was most susceptible during grain filling, moderately susceptible during tillering and least susceptible during seedling stage (Setter & Waters, 2003). However, there are few reports confirming this observation with modern barley genotypes, nor reports that have described phenological and agronomical changes in response to waterlogging. As well, it is likely that postwaterlogging growth recovery is a function of crop management, genotype, and environmental interactions, analogous to crop recovery following defoliation (Harrison, Evans, Dove, & Moore, 2011a, 2011b).

Waterlogging tolerance is a complex trait related to many morphological and physiological traits that are themselves under strong environmental influence (Zhou, Li, & Mendham, 2007). Lack of oxygen causes roots to shift energy metabolism from aerobic to anaerobic, resulting in cellular energy crises (Gibbs & Greenway, 2003). As well as tolerance to secondary metabolic compounds associated with anaerobic soil conditions (Huang et al., 2015; Pang et al., 2007), tolerant barley genotypes adapt to transient waterlogging via development of morphological mechanisms allowing plants to cope with the stress (Herzog, Striker, Colmer, & Pedersen, 2016; Hossain, Araki, & Takahashi, 2011; Kreuzwieser & Rennenberg, 2014). Morphological adaptations include adventitious roots with well-formed aerenchyma (Pang, Zhou, Mendham, & Shabala, 2004; Zhang et al., 2015). Aerenchyma (continuous gas filled channels) enhance internal diffusion of oxygen from shoots to the flooded roots, allowing roots to maintain aerobic respiration (Armstrong, 1979). Waterlogging tolerant barley genotypes such as the wild barley TAM407227 show not only higher adventitious root porosity than sensitive barley genotypes (e.g., Franklin, Naso Nijo), but also have faster development of aerenchyma under waterlogging conditions (Zhang et al., 2015).

Metabolically, tolerance mechanisms in barley include enhanced activities of glycolytic and fermentative enzymes that increase availability of soluble sugars and antioxidant defence mechanisms (e.g., superoxide radicals, hydroxyl radicals, and hydrogen peroxide) that guard against poststress oxidative damages under anaerobic conditions (Armstrong, Brandle, & Jackson, 1994; Davies, 1980; Drew, 1997; Mittler, Vanderauwera, Gollery, & Van Breusegem, 2004; Pan et al., 2019; Setter et al., 1997).

Contemporary crop breeders are now targeting genetic tolerance mechanisms including aerenchyma formation using molecular marker-assisted selection. In barley, a major QTL for aerenchyma formation under waterlogging was identified from several waterlogging tolerance genotypes

(Broughton et al., 2015; Zhang, Shabala, Koutoulis, Shabala, & Zhou, 2017; Zhang et al., 2016). This QTL was located in the same position as another QTL for waterlogging tolerance on chromosome 4H (Li, Vaillancourt, Mendham, & Zhou, 2008; Zhang et al., 2017; Zhou, 2010; Zhou, Johnson, Zhou, Li, & Lance, 2012). However, allelic differences exist in different parents, with the contribution of AF to field waterlogging tolerance ranging from 5% to 80%. A prospective allele for aerenchyma formation from a wild barley identified recently (Zhang et al., 2016) has been introgressed to a commercial variety, Macquarie (Macquarie+). This new line will be one of those examined in the present study.

In this experiment, we imposed four waterlogging treatments on six barley genotypes differing in waterlogging tolerance (two genotypes (Macquarie + and TAMF169) had the allele for AF under waterlogging stress from the wild barley). The objectives of this study were to (a) examine the impact of the timing and duration of waterlogging on grain yield and yield components, and (b) examine the contribution of the QTL for AF under waterlogging stress to mitigate yield loss.

2 | MATERIALS AND METHODS

Two experiments were conducted in 2019 at Mt Pleasant Laboratories (41°28′S, 147°08′E), Launceston, Tasmania. This region has a cool temperature climate with mean annual maximum and minimum daily air temperatures of 18.6°C and 7.4°C, respectively, and a mean rainfall of 663.4 mm per year. Daily minimum and maximum air temperatures and rainfall were recorded using an automatic meteorological station located close to the experimental site.

During the growing season, mean maximum and minimum daily air temperature were 13.9°C and 2.7°C, respectively, during the vegetative stages (Figure 1). During the reproductive stages, mean maximum and minimum air temperature were 19.3°C and 7.3°C, respectively. Cumulative rainfall was 351.3 mm during the growth season, which is below average for growing season.

Experiments were conducted with six barley genotypes including four commercial varieties: Macquarie, Franklin, Planet, Westminster, a backcross line, Macquarie+ (Macquarie/TAM407227//Macquarie), and a double haploid line from a cross of TAM407227/Franklin called TAMF169 (Table 1). Experiments were arranged in split-plot design with treatments as main plots and genotypes as subplots, each with three replications. In experiment 1, genotypes were exposed to four waterlogging treatments (Figure 2). Waterlogging treatment WL2 and a nonwaterlogged control were conducted in experiment 2. For both experiments, the leaf number at which waterlogging was applied was measured on the main stem. After each waterlogging treatment concluded, treated plots were watered near to field capacity until grain filling, after which watering was ceased. Weed control was performed from emergence to harvesting by hand hoeing. No incidence of pest or disease infection was observed in either experiment.

In experiment 1, seeds were sown in six rows in stainless steel tanks (200 cm × 100 cm × 45 cm) filled with sandy loam soil with and bottom of each tank contained 50 mm coarse gravel overlaid with drainage matting. Each row was sown with 30 seeds on 13 May 2019. Experiment 2 was conducted in a field screening facility with a waterlogging controlling system. Each genotype was sown in 1.2 m × 2 m plots with a 1.2 m row spacing of 20 cm and 30 seeds per row on 28 April 2019. The controls were sown in well-drained beds. Three replicates were applied for both waterlogging treatment and controls.

For both experiments, three replicate tanks (plots) were used for each treatment. Plants were fertilized with 24 kg/ha of YaraMila Complex (12%N:11%P_2O_5:18% K_2O, Yara

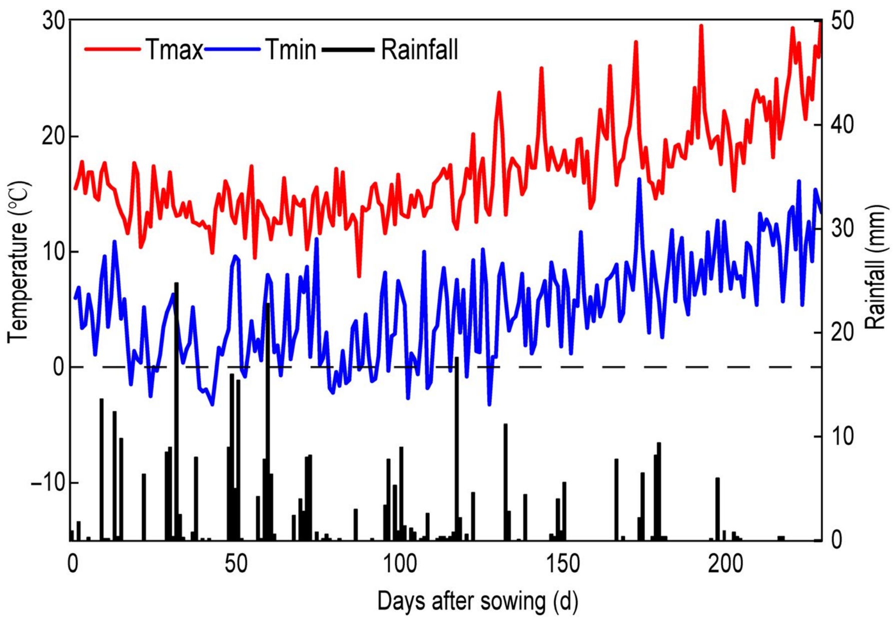

FIGURE 1 Air maximum and minimum temperature and rainfall over the growing season in Launceston, Tasmania, Australia in 2019

TABLE 1 Barley cultivars used in the study, year of release, pedigree, and commercializing organization

Genotype	Pedigree	Source
Macquarie	Alexis/Gairdner//Gairdner	Commercial variety released by the University of Tasmania
Macquarie+	Macquarie/TAM407227//Macquarie[3]	A backcross lines with the background of Macquarie and the waterlogging tolerance QTL from a wild barley, by the University of Tasmania
Planet	Tamtam/Concerto	A commercial variety released by Seed Force Pty Ltd
Franklin	Shannon/Triumph	A commercial variety released by the University of Tasmania
Westminster	NSL97-5547/Barke	A commercial variety released by GrainSearch
TamF169	TAM407227/Franklin	A doubled haploid line from the cross between TAM407227 and Franklin, by the University of Tasmania

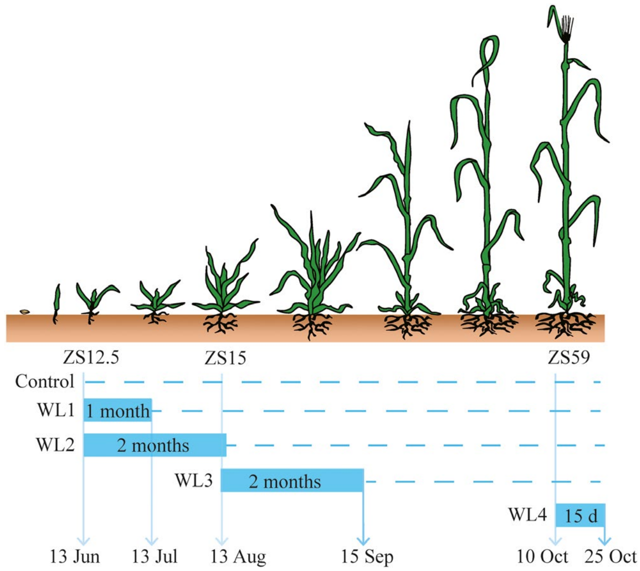

FIGURE 2 Diagram indicating the start and end date of each waterlogging treatment. WL1: waterlogging exposed at ZS12.5 for 1 month; WL2: waterlogging exposed at ZS12.5 for 2 months; WL3: waterlogging exposed at ZS15 for 2 months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 treatment was not conducted on Franklin and Westminster

Company) as basal. During the growth periods, all treatments were top-dressed with equal amounts of 50 kg/ha of YaraMila Complex at jointing (ZS32, Zadoks, Chang, & Konzak, 1974) and booting (ZS45), respectively. No signs of nutrient deficiencies were observed. The soil in all tanks was consistent, homogenously mixed and had soil properties of pH = 6.8; total N = 31.18 mg/kg; total p = 30.75 mg/kg, total K = 98.63 mg/kg, field capacity = 0.289 g/g, lower limit = 0.096 g/g, and bulk density = 1.02 g/cm^3.

2.1 | Waterlogging treatments

For experiment 1, a water tray was used to supply water to the bottom of each tank (Figure 3). The water level of each container was maintained at 75 mm depth by fitting a float valve to a reservoir. Excess water from rainfall flowed back to the reservoir and out an overflow. Any water lost from the plant containers through evapotranspiration that reduced the water level below 75 mm was resupplied by the reservoir to maintain the water level. Control plots were watered near to field capacity until grain filling. Waterlogging was achieved by raising the reservoir above the soil surface such that the water level increased to 400 mm and the soil was completely saturated (lower panel in Figure 3).

2.2 | Measurements

2.2.1 | Phenology

Crop phenology was measured every 2 weeks following the Zadoks stage (Zadoks et al., 1974).

2.2.2 | Shoot biomass

For each treatment, three plants were selected for destructive biomass measurement before and after waterlogging treatments. At maturity, shoot biomass was harvested from three plants in each pot and separated into stem, leaf, and spike. Samples were then oven dried at 65°C for at least 48 hr until constant weight.

To determine the effect of waterlogging on the growth during and after waterlogging, the following indices were calculated for each waterlogging treatment (Arduini, Baldanzi, & Pampana, 2019).

The relative biomass (RB) is calculated as:

$$\mathrm{RB} = \frac{B_W}{B_C} \times 100\%$$

where B_w is the dry biomass at the end of waterlogging treatment, and B_c is the dry biomass of control at the same time.

2.2.3 | Grain yield and yield components

At maturity, plants in the middle three rows of each plot (90 plants per treatment) were selected for determination of grain yield and yield components. Spike number was enumerated in each plot and recorded prior to harvest. All spikes were manually harvested, threshed, and weighed to calculate grain yield. One thousand random kernels from each harvested grain were weighed to calculate a 1000-kernel weight. Grain moisture was measured using a Grain Analyser (InfratecTM 1241, Foss, Denmark). Grain yield and 1000-kernel weight were adjusted to 13% moisture. The average number of kernels per spike was enumerated from 30 spikes.

Grain size parameters, including 1,000 kernel weight, grain length, width, and thickness were measured using SeedCount SC5000 (Next Instruments, Condell Park, NSW, Australia) and a digital balance.

TABLE 2 Analysis of variance (ANOVA) of grain yield (GY), spike number (S), kernels per spike (KS), 1,000 kernel weight (KW), grain length (van der Moezel et al.), grain width (GW), grain thickness (GT), and shoot biomass (SB)

ANOVA	Variety(V)	Treatment (T)	V * T
GY	37.3***	242.92***	12.2***
S	31.76***	167.96***	28.88***
KS	113.86***	259.43***	107.11***
KW	519.48***	210.00***	214.25***
GL	250.66***	113.07***	100.61***
GW	1,104.96***	836.20***	1,011.58***
GT	1,288.52***	929.8***	967.38***
SB	44.10***	8.37*	6.04***

***Significant at $p < .001$.

*Significant at $p < .05$.

2.3 | Statistical analysis

Data were analyzed using two-way (treatment and genotype) analysis of variance with SAS9.2 (SAS Institute Inc.). Means of each treatment and each genotype were compared based on a least significant difference (LSD) at a probability level of 0.05.

3 | RESULTS

3.1 | Grain yield

In experiment 1, the average grain yield reduction for WL1, WL2, WL3, and WL4 across genotypes was 35%, 46%, 52%, and 70% compared with the control, respectively (Table 2; Figure 4a). Yield loss in the waterlogging-susceptible variety Franklin was 47% in WL2 and this genotype died completely in WL3. Yield loss in the waterlogging-tolerant genotype (Macquarie+) was 17% and 21% in WL1 and WL3, respectively, which was lower than other genotypes. In experiment 2, the average grain yield reduction for WL2 was 25%–59% (Figure 4b). Franklin and Planet showed the greatest yield reduction, while TAMF169 and Macquarie+ were the least reduced by waterlogging. Yield loss in Macquarie+ was 17%–21% in comparison with 43%–52% in Macquarie under continuous one or 2-month waterlogging conditions in experiment 1 and was 18% (Macquarie+) versus 38% (Macquarie) in experiment 2.

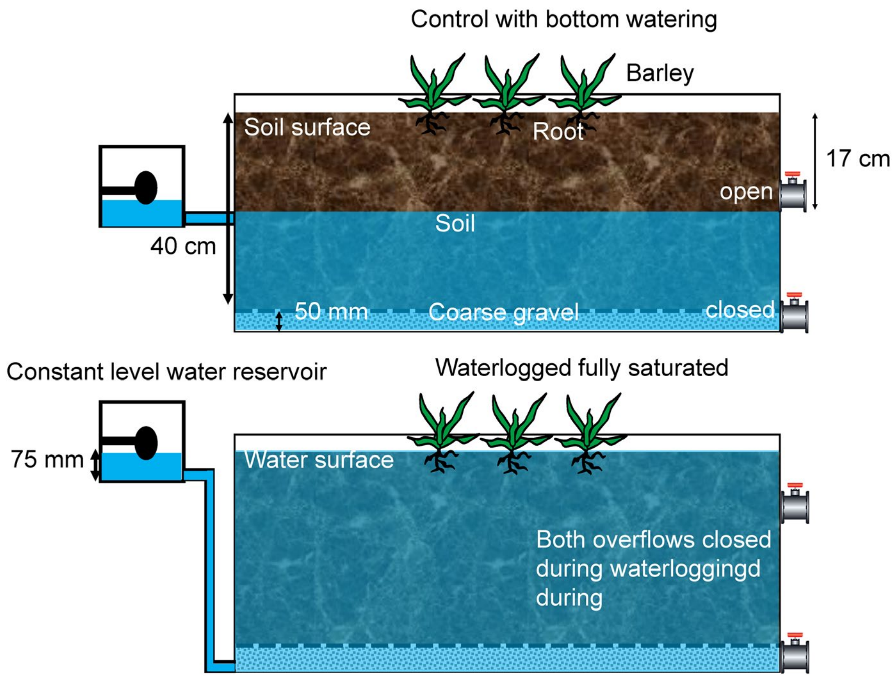

FIGURE 3 Location of water reservoir relative to the plant tanks to achieve waterlogging. Upper diagram: watering of control plants; lower diagram, waterlogged treatments

3.2 | Yield components

Spikes per m^2 and kernels per spike were reduced by all waterlogging treatments (Table 2; Figure 5). WL1-3 reduced spikelets per m^2. No tiller death was observed for WL4 as this treatment was applied after ear emergence (spikelets per m^2 were not affected). WL2 caused the highest spike number reduction across genotypes (average decline of 37%). All treatments reduced kernels per spike except WL1 and WL2 for Westminster. WL4 caused the greatest reduction in kernels per spike for all genotypes (except TAMF169) by increasing numbers of infertile spikelets (19%). When waterlogging treatments (WL1, WL2, WL3) occurred relatively early in crop phenology (ZS12.5-ZS15), 1000-kernel weight was not affected or even increased in some barley genotypes (e.g., Franklin, Macquarie+ and Westminster) due to reduced number of spikes per m^2 and kernels per spike. In contrast, waterlogging in later crop development stages (WL4) reduced 1000-kernel weight by more than 50%. These results indicate that yield penalty was primarily associated with (a) reduced tillering when waterlogging was applied at early growth stages (WL1-3) or (b) with reduced spikelet fertility and grain filling when waterlogging was applied at ear emergence (WL4). An extreme example is that none of the Franklin plants survived under WL3 (leading to 100% yield reduction).

To better understand how waterlogging affected 1,000 kernel weight, further measurements were conducted on

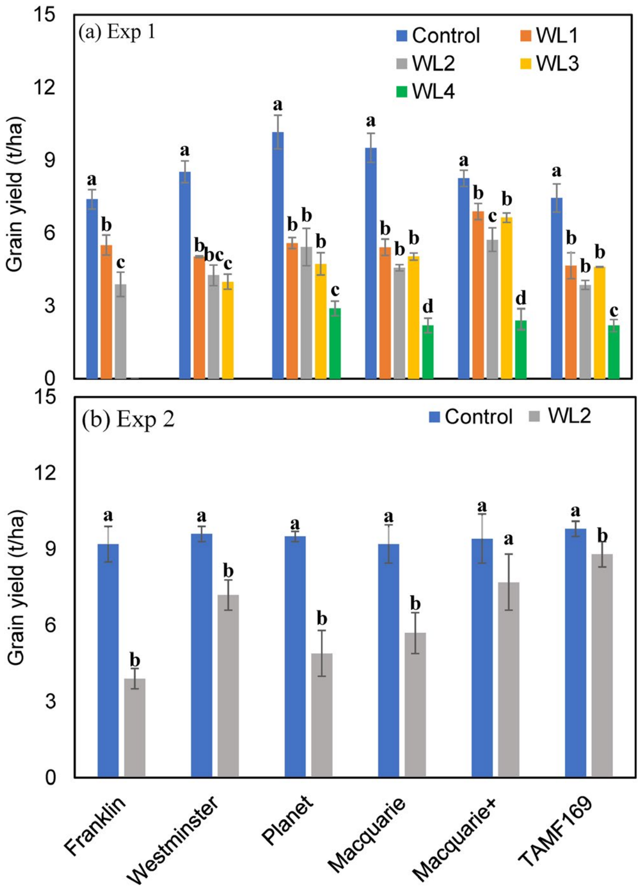

FIGURE 4 Effect of waterlogging treatments on grain yield. WL1: waterlogging exposed at ZS12.5 for 1 month; WL2: waterlogging exposed at ZS12.5 for 2 months; WL3: waterlogging exposed at ZS15 for 2 months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 treatment was not conducted on Franklin and Westminster. Vertical bars indicate ± standard error of the mean. The different letters mean significant differences in each barley genotype among or between different treatments

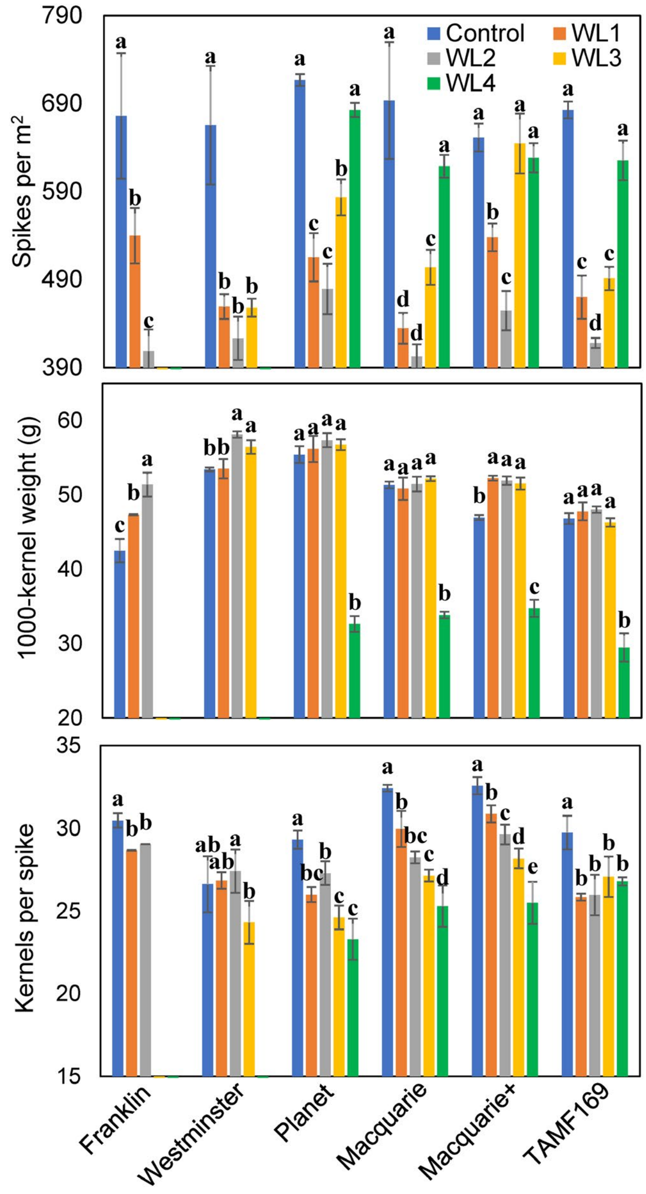

FIGURE 5 Effect of waterlogging treatments on yield components. WL1: waterlogging exposed at ZS12.5 for 1 month; WL2: waterlogging exposed at ZS12.5 for 2 months; WL3: waterlogging exposed at ZS15 for 2 months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 treatment was not conducted on Franklin and Westminster. Vertical bars indicate ± standard error of the mean

grain size. Waterlogging reduced grain length for all barley genotypes except for Franklin, which showed a slight increase in WL1 (5%) and WL2 (10%) (Figure 6). In contrast, all waterlogging treatments except WL4 increased grain width and grain thickness, which contributed unchanged or even increased grain weight despite decreased grain length. WL4 reduced grain width across genotypes, with an average reduction of 20%. Grain thickness in genotypes Planet, Macquarie, Macquarie+, and TamF169 was reduced, decreasing by 5%, 11%, 13%, and 14%, respectively, compared with the controls. The combination of reduced grain width and thickness is the major contributor for the huge reduction 1,000 grain weight in WL4.

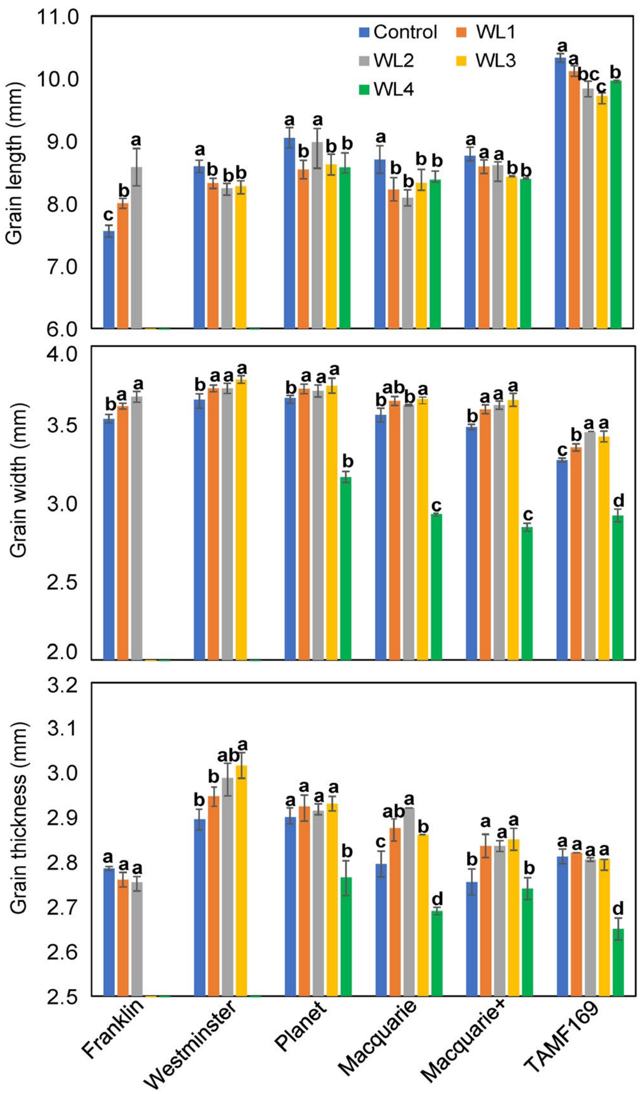

FIGURE 6 Grain dimensions of six barley genotypes in response to waterlogging. Vertical bars indicate ± standard error of the mean. WL1: waterlogging exposed at ZS12.5 for 1 month; WL2: waterlogging exposed at ZS12.5 for 2 months; WL3: waterlogging exposed at ZS15 for 2 months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 was not conducted on Franklin or Westminster

3.3 | Shoot biomass

Franklin had the greatest capacity to recover from WL1; RB of WL1 was 56% after waterlogging concluded but recovered to 79% by harvest (Figure 7). Westminster had the greatest capacity to recover from WL2; RB of Westminster after waterlogging was 29% (Figure 7) and 78% by harvest. Macquarie+ had the greatest capacity to recover from WL3, for which there were no significant effects of waterlogging.

Across genotypes, the average biomass reduction at maturity for WL1, WL2, WL3, and WL4 was 28%, 41%, 52%, and 55%, respectively (Figure S1). Generally, the largest reduction in maturity biomass caused by waterlogging was in treatment WL4 on individual genotypes, with biomass reductions ranging from 50% to 68%. The main effect of waterlogging on shoot biomass was on dry spike weight and to a lesser extent dry stem and leaf weight, particularly WL4. Franklin did not recover from WL3 (WL4 was not conducted on Franklin and Westminster).

3.4 | Effects of waterlogging stress on phenology (crop growth stages)

Phenology at the end of each waterlogging treatment was delayed relative to the controls (Figure 8). The greatest delay occurred in the WL2 treatment and the least in WL4 (WL4 began after ear emergence had no effect on phenology). Franklin was delayed the most by waterlogging, while TAMF169 and Macquarie+ were the least delayed. Maturity dates were delayed by 8–15 days by waterlogging at early growth stages across genotypes (Figure 8). In contrast, WL4 resulted in premature, and maturity dates were 5–8 days earlier in WL4 compared with controls.

4 | DISCUSSION

The purpose of this study was to examine the influence of waterlogging at different phases of phenology, and the mechanisms and the extent to which these effects influenced yield. The contribution of AF to mitigation of yield reduction under waterlogging and ability to recover from waterlogging stress were also examined.

4.1 | Physiological mechanisms implicit to crop recovery from waterlogging

Waterlogging caused transient reductions in biomass accumulation, but the final impact on grain yield depended on the capacity of plants to recover after waterlogging (de San Celedonio, Abeledo, Mantese, & Miralles, 2017). In winter

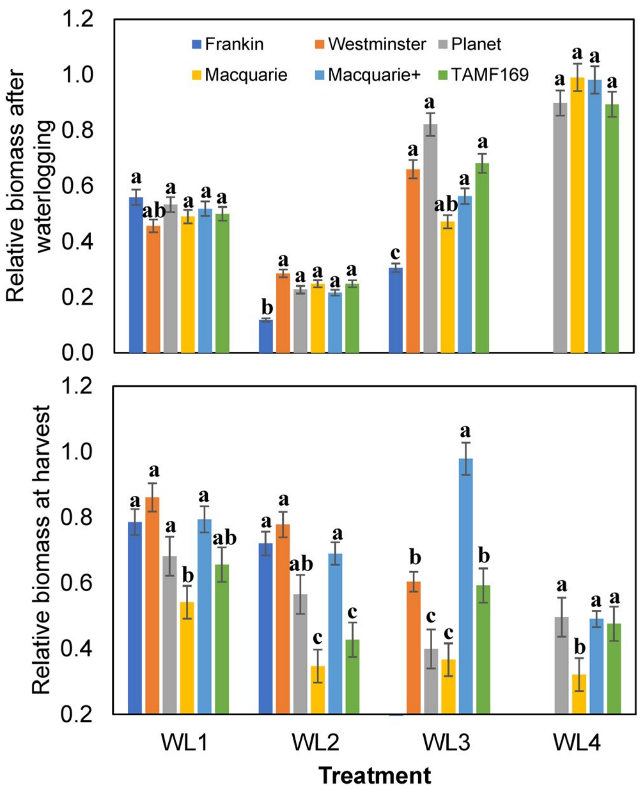

FIGURE 7 Biomass either at the end of waterlogging (top panel) or at harvest (bottom panel) relative to that of respective controls at harvest of each genotype under different waterlogging treatments. WL1: waterlogging exposed at ZS 12.5 for 1 month; WL2: waterlogging exposed at ZS12.5 for 2 months; WL3: waterlogging exposed at ZS 15 for 2 months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 was not conducted on Franklin or Westminster

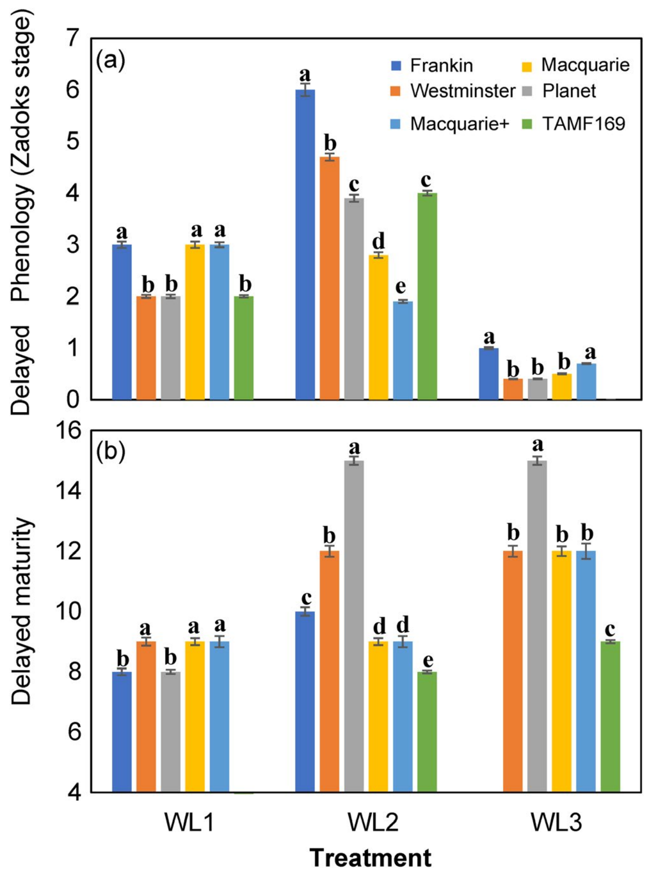

FIGURE 8 Delay in phenology at the end of waterlogging (top panel) or at harvest (bottom panel) relative to phenology of respective controls. WL1: waterlogging exposed at ZS 12.5 for 1 month; WL2: waterlogging exposed at ZS12.5 for 2 months; WL3: waterlogging exposed at ZS 15 for 2 months

cereals, survival of root apices and lateral root initials under waterlogging and the restoration of tillering upon drainage are considered crucial plant traits to ensuring recovery (Herzog et al., 2016; de San Celedonio et al., 2016). The former allows plants to resume root growth and supply shoots with nutrients, while the latter helps replace tillers that have died or developed minor inflorescence under waterlogging (Arduini et al., 2019). In this study, Franklin showed greater recovery from short-term waterlogging treatment (WL1) compared with other barley genotypes. WL1 only caused a 26% yield loss in Franklin compared with around 40% in the other sensitive genotypes. This was because Franklin has a longer vegetative growth duration, allowing plants a longer period to produce more tillers and recover. This capacity to recover decreased the later waterlogging was imposed in the phenological cycle. Previous studies have shown that the capacity of barley to recover shoot biomass after waterlogging is related to genotypic and environmental propensity to produce new tillers (Robertson, Zhang, Palta, Colmer, & Turner, 2009; de San Celedonio et al., 2016). Thus, when barley plants are waterlogged late in their lifecycle (e.g., beginning of stem elongation), they are not able to produce new tillers and compensate for the lost shoot biomass caused by waterlogging (de San Celedonio et al., 2017).

4.2 | Effects of waterlogging on phenology and implications for yield

Waterlogging treatments (WL1-3) delayed maturity (Figure 8) for all genotypes, with WL3 having the greatest effect on phenology and on biomass, suggesting that imposition of waterlogging later in the crop lifecycle has the greatest implications for yield. Such yield penalization can occur either via reductions in leaf area and canopy development leading to lower biomass accumulation (WL1-3) or in yield components if waterlogging is imposed very late in the crop lifecycle (WL4).

In barley, flowering date is primarily a function of temperature, photoperiod, and vernalization (Liu et al., 2020). The rate of leaf emergence and final leaf number determine the duration of the period between emergence and anthesis (Alzueta, Abeledo, Mignone, & Miralles, 2012). Here, waterlogging at early growth stages (WL1-3) inhibited leaf appearance rate and reduced final leaf number, delaying flowering and maturity by 7–15 days. This may be linked with oxygen deficit induced denitrification with the consequent loss of nitrate in waterlogged soils. Since nitrate is essential for physiological function, growth is quickly affected. Here, it was shown that leaves of most genotypes yellowed around 5 days after waterlogging, and the extent of yellowing increased with increasing waterlogging durations. Early yellowing of basal leaves during waterlogging coincides with lower photosynthetic rate (Hossain et al., 2011), lower photosynthesis then likely caused lower shoot growth. The phenology of the waterlogging-tolerant TAMF169 and Macquarie+ was the least delayed by waterlogging, indicating that ability to avoid phenological delay may correlate with greater waterlogging tolerance, though more evidence is needed to support this claim. As such, we call for further work on the relationships between waterlogging tolerance and the impact of waterlogging on phenology.

4.3 | Relationship between yield and yield components

Regardless of waterlogging treatment or genotype, waterlogging reduced yield (Figure 4). Such reductions were mainly caused by fewer spikes per m^2 when waterlogging occurred in early phenology. WL1-3 had similar effects on yield and yield components, with reduction in spikes per m^2. This is likely to be due to the growth stage when waterlogging treatments were imposed. WL1-3 were applied prior to/at tillering stages (ZS12.5 and ZS15); all three treatments caused reductions in tiller numbers (data not shown), such that spike numbers were reduced at the end of waterlogging for all treatments except WL4. The number of fertile spikes at maturity represents the balance between tiller appearance rate (Alzueta et al., 2012) and tiller mortality (Baethgen, Christianson, & Lamothe, 1995; García del Moral & García del Moral, 1995). Thus, reduced spike numbers in WL1-3 could be attributed to lower tillering under waterlogging, similar to effects of nutrient deficiency (Alzueta et al., 2012; Masoni et al., 2016) and water deficit on tillering (Cossani, Slafer, & Savin, 2009).

Reduced spike length caused fewer kernels per spike under waterlogging (Arisnabarreta & Miralles, 2006; García del Moral & García del Moral, 1995). Westminster was the only genotype that did not show a reduction in kernels per spike under waterlogging treatments WL1-3. Waterlogging induced a higher grain weight compared with controls in Franklin, Westminster, and Macquarie+. The increase in grain weight under waterlogging was attributed to increased grain length in Franklin, and grain width and thickness for Westminster and Macquarie+ (Figure 6, Figure S2). Fewer kernel numbers per spike caused by waterlogging could result in more photosynthate and stored assimilate for grain growth and kernel weight, compensating for detrimental effects of waterlogging on other yield components to some degree. Similar effects have been observed for wheat defoliated in vegetative stages in which more assimilate is partitioned to kernels of grazed crops (Harrison, Evans, Dove, & Moore, 2011a, 2011b).

4.4 | Late stage waterlogging caused the greatest yield reduction

The largest grain yield penalty occurred when waterlogging was applied close to heading, even though the duration of waterlogging was very short (WL4; Figure 4). This finding is in line with previous results (de San Celedonio et al., 2014; Setter & Waters, 2003). Late stage waterlogging stress coupled with high temperature (mean daily temperature >30°C) may cause greater damage to yield reduction in some regions, for example, wheatbelts located in the lower and middle reaches of Yangtze River in China (Wu et al., 2013), the eastern Gangetic plains of India (Tiwari et al., 2012), but this is not the case in our region because maximum daily temperatures between flowering and maturity reached only 19.9°C; this temperature is unlikely to have damaged grain growth or filling. In this study, lower grain yield under waterlogging was linked with lower grain weight and, to a lesser extent, lower kernels per spike. In this treatment, waterlogging caused premature leaf senescence of all genotypes, which led to lower stomal conductance (Araki, Hamada, Hossain, & Takahashi, 2012) and photosynthetic rate (Hossain et al., 2011). Waterlogging stress during grain-filling period may also reduce carbon assimilation rates and result in lower remobilization of culm reserves (Alessandro Masoni, Ercoli, Mariotti, & Pampana, 2008; Schnyder, 1993).

4.5 | An aerenchyma gene improved waterlogging tolerance

Our results show that most currently available Australian barley genotypes are intolerant to waterlogging. It is thus crucial that further scientific endeavour is undertaken to develop more waterlogging-tolerant genotypes that alleviate yield losses caused by waterlogging.

Our previous studies have identified QTL controlling root AF under waterlogging stress, which is one of the major mechanisms for waterlogging tolerance in barley (Zhang et al., 2016). This gene was introgressed into a commercial variety Macquarie through repeated backcrossing and the new line Macquarie+ (Macquarie background with AF QTL) was included in this experiment. After 2 weeks of waterlogging, Macquarie+ showed a much greater proportion of aerenchyma in its roots compared with Macquarie. Of all genotypes examined, Macquarie+ was the most tolerant to waterlogging (Figure 4a,b); evidenced by higher numbers of spikes/m^2 and to a lesser extent maintenance of grain weight under waterlogging (Figure 5). The QTL for AF mitigated around 23% yield loss under waterlogging stress, suggesting that the QTL is effective in improving waterlogging tolerance of commercial varieties and can be used in breeding programs.

5 | CONCLUSIONS

Here, we examined the impacts of waterlogging on susceptible and tolerant waterlogging barley varieties. We also examined how the timing of waterlogging relative to phenology impacted on yield. Our analysis suggests that waterlogging close to heading is the most susceptible period, with yield losses primarily attributed to reductions in spikelet fertility and grain weight. Yield loss caused by waterlogging at earlier growth stages was mainly a consequence of reduced spike number and to a lesser extent kernels per spike. Early waterlogging combined with long duration genotypes (e.g., Franklin) may be conducive to tillering in some situations and greater recovery, provided the time to initiate new tillers after waterlogging is sufficient before flowering. When waterlogged at a late stage, the phenology of waterlogging-tolerant genotypes was less delayed compared to waterlogging susceptible genotypes. We also showed that AF was conducive to waterlogging tolerance through lower effects of waterlogging on phenology and relatively lower yield penalty.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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